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Enhanced Tumor Targeting by an Intratumoral Injection of Colloidal Chromic ^{32}P in Two Human Tumors (AsPC-1 Pancreas and Ls174T Colon) in Nude Mice

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Background and Objectives: To find the mechanisms of the ongoing clinical trials in intralesional colloidal chromic ^{32}P (^{32}P-CP) brachytherapy, the cellular uptake of ^{32}P-CP, changes in tumor interstitial fluid pressure (TIFP), and tumor blood flow (TBF) using two (AsPC-1, Ls174T) human tumors were measured.

Methods: After exposure to ^{32}P-CP using exponential and plateau-phase cells, cells were trypsinized at various time intervals, then measured for the levels of radioactivity using a γ-counter. Also measured were TIFP using the WIN technique and TBF with laser Doppler flowmetry.

Results: The plateau growth-phase of both tumors showed the maximal uptake of ^{32}P-CP at ~100 min. TBF decreased within 10 min after an intratumoral (i.t.) injection of ^{32}P-CP, and reached 75% of control value by 1 h.

Conclusions: If ^{32}P-CP was introduced i.t., it maintained highly efficient tumor targeting, mainly due to two physiological mechanisms: the high adherence of ^{32}P-CP to the infused regions and the reduction in TBF by this therapeutic colloid. *J. Surg. Oncol. 1999;70:161–166.*

KEY WORDS: tumor targeting; colloidal chromic ^{32}P; tumor hypertension; tumor interstitial fluid pressure; tumor blood flow

INTRODUCTION

During the last 50 years, colloidal chromic ^{32}P (^{32}P-CP) has been applied for the treatment of intracavity malignancies. The rationale for the clinical use of ^{32}P-CP is the absence of substantial leakage of the radioactivity into regional nodes and liver as well as the lack of chromosomal damage in nonlesional regions [1,2]. Recently, Condra et al. [3] recommended the inclusion of an intraperitoneal ^{32}P-CP in prospective trials of adjuvant and consolidative therapy for ovarian carcinoma. Levine et al. [4] reported unsuccessful tumor targeting when this therapeutic colloid was infused via the portal or hepatic artery. We believe that their failure in tumor targeting of ^{32}P-CP via regional infusion was due to the hypertension in solid tumors [5–7].

During the course of active studies of radiolabelled monoclonal antibodies (MAbs) for the last 2 decades, it was found that MAbs only accumulated to <1% of the cancer patients' dosages. The low uptake and short retention of radiolabelled MAbs in tumors limited total doses of radiation to <20 Gy in most clinical trials. One of the causes for such discouraging observations may have been due to the elevated tumor interstitial fluid pressure (TIFP) that caused a physiological barrier in the delivery of macromolecules into solid tumors [5–7]. To overcome this physiological barrier, a large volume of material via an intratumoral (i.t.) injection was required, usually too much to be viable. The alternative to this was to decrease TIFP without any modification of the sys-

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temic blood pressure or microvascular pressure [8–10]. When a therapeutic agent was mixed with a large quantity of fluid directly infused into the center of a tumor, it would increase the pressure at the core of the tumor relative to the surroundings. Consequently, the drug would spread along with an artificially induced pressure gradient by convection from the core through the surrounding region to the periphery.

Recently, an i.t. infusion of dexamethasone (dexth) was applied to overcome elevated TIFP in pancreatic cancer patients, but no measurements of TIFP were performed [11,12]. In the state of severe malignancy of nonresectable pancreatic cancer, Order et al. [11,12] treated these patients using i.t. ^{32}P-CP brachytherapy in conjunction with an i.t. injection of macroaggregated albumin (MAA). Greater than 85% of the ^{32}P-CP was found to be deposited in the pancreatic carcinoma after the first i.t. infusion of MAA + ^{32}P-CP [13]. It was then proposed that ^{32}P-CP had been retained intratumorally due to MAA-induced transient blockage in the outflow of the tumor vasculature. However, our recent preclinical observations using three different tumor vasculature, H4IIE rat hepatoma, MCaIV murine adenocarcinoma, and FSaII murine fibrosarcoma conflicted with Order's observation that an i.t. infusion of MAA remarkably increased tumor targeting [14]. For better understanding of the ongoing clinical trials in intralesional ^{32}P-CP brachytherapy, the cellular uptake of ^{32}P-CP and changes in physiological parameters—mainly TIFP and tumor blood flow (TBF) after infusions of ^{32}P-CP, MAA, albumin, or dexth—were studied using two human tumors, AsPC-1 pancreatic carcinoma and Ls174T colon adenocarcinoma, in nude mice.

MATERIALS AND METHODS

Animals and Tumors

Female 8–10 week-old, nude mice (purchased from the Cox Animal Facility, Massachusetts General Hospital, Boston, MA) bearing human tumor xenografts (AsPC-1 human pancreatic carcinoma, Ls174T human colon adenocarcinoma) were utilized. Animals were kept under pathogen-free conditions in the vivarium, maintained at 25 ± 3°C. The institution's guidelines for the care and use of laboratory animals were followed. The mice were allowed food and water ad libitim. Frozen AsPC-1 and Ls174T tumor cells (purchased from ATCC, Rockville, MD) were thawed, cultured, and grown in vitro. Single-cell suspensions were prepared using 0.25% trypsin solution. Approximately 1×10^6 viable cells suspended in 50 μl of dMEM medium were injected subcutaneously (s.c.) into the right thighs of mice. Experiments were carried out when the tumor volume was between 100 and 2,000 mm^3. Tumor volumes were calculated using the formula $V = 0.4 \times ab^2$, where a and b were the longer and shorter diameters of the tumor, respectively [15].

In Vitro ^{32}P-CP Cellular Uptake Measurements

Cells were plated in 24 multiwell plates, then incubated to grow in either exponential or plateau growth-phase. After exposure to 10 μCi of ^{32}P-CP (Mallinckrodt Medical, Inc., St. Louis, MO), cells were trypsinized at various time intervals. The radioactivity was then counted using a γ-counter [14].

Anesthesia

Mice were anesthetized with ketamine (90 mg/kg) and xylazine (9 mg/kg) via intramuscular (i.m.) route. Mice were placed on a heating pad to keep the body temperature ~37.5°C and maintained by monitoring their rectal temperature using a Type-T thermocouple and BAT-10 thermometer (Physitemp Inc., Clifton, NJ) [9,10].

Measurements of TIFP

TIFP was measured with the wick-in-needle (WIN) technique using 23 gauge needles with a side hole 2 mm from the tip. Measurements were made by introducing WIN needles into the central regions of the tumors using a Mac Lab/4 analog digital system (ADInstruments, Milford, MA) linked to a Macintosh computer [9,10]. However, for the measurements of TIFP in tumor volume of <100 mm^3, the WIN needles without a side hole were used.

Measurements of TBF

TBF was measured using the Laserflow Blood Perfusion Monitor 403A (Vasamedics, St. Paul, MN) with a 0.8 mm-diameter laser Doppler needle probe. Briefly, a small hole was made in the tumor using a 23 gauge needle. A needle probe was inserted into the tumor center, then slightly withdrawn to ensure that there was no compression of the tumor under the probe tip. The electrical signals of flow, volume, and velocity from the laser Doppler system were digitally processed using a Mac Lab/4 analog digital system linked to a Macintosh computer with output voltage ranging from 0 to 2.5 V. At the end of the experiments, the zero-flow signal was measured after sacrificing the animals with an overdose of anesthesia [9,10].

Statistical Evaluation

All measured values in Tables I–IV and Figures 1 and 2 are shown as the median of each group at different time points for the nonparametric statistic. In Table I, the individual median value was derived from six independent samples per time (i.e., 1 min, 10 min, 25 min, etc.). Cellular uptake in various environments (i.e., plastic alone, exponential-growth phase, etc.) after various incubation times ranging from 10 min to 180 min was

TABLE I. Cellular Uptake (Level of Radioactivity) After Various Incubation Times With 10μCi of ^{32}P-CP*

	1 min	10 min	25 min	60 min	100 min	180 min
I. Plastic alone (24-well plate)	0.10	0.06[a]	0.09[a]	0.08[a]	0.09[a]	0.10[a]
II. Exponential-growth phase						
Ls174T human colon adenocarcinoma	1.35[b]	1.52[a,b]	2.15[b,c]	2.10[b,c]	2.50[b,c]	2.55[b,c]
III. Plateau-growth phase						
Ls174T human colon adenocarcinoma	2.12[b]	3.13[b,c]	4.04[b,c]	4.54[b,c]	5.45[b,c]	5.60[b,c]
AsPC-1 human pancreatic adenocarcinoma	0.21[d]	0.75[a,d]	1.71[b,c]	2.73[b,c]	3.66[b,c]	3.86[b,c]

*Median value; unit: μCi per aliquot; n = 6 independent samples per time. For each comparison, the Mann-Whitney two tailed test was applied.
[a]vs. 1 min, not significant.
[b]vs. plastic alone, $P < 0.05$.
[c]vs. 1 min, $P < 0.05$.
[d]vs. plastic alone, not significant.

TABLE II. Elevated Tumor Interstitial Fluid Pressure (TIFP) in Mice Bearing Human Tumor Xenografts

	Skeletal muscles	Ls174T	Ls174T	AsPC-1
Strain	Nude	Nude	SCID	Nude
Tumor lines	—	Colon	Colon	Pancreas
TIFP (mmHg)	−1.5	10.3	18.5	21.3
Range	[−4.5, 0]	[7.8, 18.5]	[7.5, 34.5]	[8.1, 34.1]
No. of mice	10	10	10	10
Tumor volume (mm^3)	—	245	230	220

compared to the median value at 1 min, based on their environments, by a two-tailed Mann Whitney U-test. Then, at each time point (i.e., 1 min, 10 min, 25 min, etc.), the Kruskal-Wallis test was applied across environments for one-way analysis of variance. In Tables III and IV, relative changes were determined individually for each mouse based on pretreatment values. For each comparison, Wilcoxon's two-sample test was applied. In Figures 1 and 2, median TIFP was derived from the same seven mice to 7 days. Wilcoxon's two-sample test was applied for comparison to the control value. *P* values smaller than 0.05 were considered significant.

RESULTS

In Table I, the median values of passive adsorption of this radioactive colloid by 24 multiwell plates (control group: no plating cells in wells) were <0.1 μCi (or <1%) per aliquot. These uptake levels remained statistically constant during the 3 h after incubation of 10 μCi of ^{32}P-CP. Maximal uptake occurred in both exponential and plateau growth-phases at ~100 min postincubation of this colloid. The uptake of ^{32}P-CP in the exponential growth-phase of Ls174T was approximately two times less than that of plateau growth-phase during 3 h of incubation ($P < .01$). The plateau growth-phase of both Ls174T and AsPC-1 tumors showed that the maximal uptake rate was reached at ~100 min. During the initial short period of treatment with ^{32}P-CP, the cellular uptake rates in Ls174T tumors varied from ~3–4 times (at 10 min and 25 min) to ~10 times (at 1 min) more than those in AsPC-1. The maximal uptake (at 100 min) in Ls174T was ~1.5 times higher than that in AsPC-1 ($P < 0.01$). The acute cytotoxic effects of either ^{32}P-CP (0–100 μCi per well) or MAA (10^4–10^6 particles per well) was examined by using a trypan blue exclusion assay by incubating cells for the indicated time points (i.e., 1, 3, and 24 h). We did not observe any time-dependent or dosage-dependent effects of either ^{32}P-CP or MAA on cell number and cell viability. In animals bearing Ls174T tumors, there were no differences in tumor retention of radioactivity or growth inhibition induced by ^{32}P-CP in the absence and presence of MAA (data not shown).

The two human tumor xenografts displayed elevated TIFP compared with interstitial fluid pressure (IFP) in the skeletal muscle of the same nude mice (Table II). TIFP values in Ls174T tumors varied depending on the strain of mice. For example, the median TIFP in nude mice was 10.3 mmHg, and that in SCID mice was 18.5 mmHg. The median TIFP in AsPC-1 tumors was 21.3 mmHg. To clarify the physiological responses following an i.t. infusion of ^{32}P-CP ± MAA, changes in TIFP were monitored using our tumor models after various infusions: ^{32}P-CP, MAA, human albumin (same concentration as the dosage of MAA), and dexth. In Ls174T tumors, TIFP returned to the original level within 1 h after an i.t. infusion of various substances (Table III). We observed that TIFP significantly reduced to ~60% ($P < 0.01$) of the original levels 48 h after an i.t. infusion of ^{32}P-CP. Although no remarkable dexth-induced reduction in TIFP was observed within 48 h, TIFP reduced to ~40% ($P < 0.05$) of the original levels 5 days after an i.t. infusion of dexth, then returned to the original level in 7 days (Fig. 1). In AsPC-1 tumors, the trend of changes in TIFP by ^{32}P-CP, albumin, and dexth was similar to results from Ls174T tumors. Interestingly, as shown Figure 2, an i.t. infusion of MAA slightly dropped TIFP within 30 min, but did not significantly reduce TIFP until days 2–7. We also observed that TIFP in AsPC-1 tumors significantly increased with tumor volume from 100 mm^3 to 2,000 mm^3 (data not shown).

Using Laser Doppler flowmetry, an i.t. injection of

TABLE III. Relative Changes in TIFP of Ls174T Tumors in Nude Mice After an Intratumoral Infusion of Various Substances*

Time	0.9% saline	^{32}P-CP[a]	MAA[a]	Albumin[a]	Dexamethasone[a]
0 min	1.00	1.00[b]	1.00[b]	1.00[b]	1.00[b]
5 min	1.30[c]	1.31[b,c]	2.16[c,d]	1.12[b,e]	1.27[b,c]
10 min	1.15[e]	1.19[b,e]	1.95[c,d]	0.98[b,e]	1.18[b,e]
1 hr	1.05[e]	0.96[b,e]	1.56[c,d]	0.92[b,e]	1.10[b,e]
24 hrs	0.99[e]	0.82[c,d]	0.93[b,e]	0.90[b,e]	1.05[b,e]
48 hrs	1.02[e]	0.64[c,d]	1.30[b,e]	0.99[b,e]	0.90[b,e]

*Median value; n = 7 mice per group.
[a]Treated tumors were compared to the control (0.9% saline group) at each time point, and the TIFPs obtained at various time points were compared to their initial values (0 min) in the same tumors. For each comparison Wilcoxon's two-tailed test was applied.
[b]vs. saline, not significant.
[c]vs. 0 min, $P < 0.05$.
[d]vs. saline, $P < 0.05$.
[e]vs. 0 min, not significant.

TABLE IV. Changes in TIFP and TBF in Mice Bearing AsPC-1 Tumors After an Intratumoral Injection of Either ^{32}P-CP or Saline*

	–5 min	0 min	1 min	5 min	10 min	30 min		24 h
TIFP (mmHg)								
^{32}P-CP	22.0	22.0	34.7	15.4	15.4	16.1		13.5
vs. saline	NS	NS	NS	$P < 0.05$	$P < 0.05$	$P < 0.05$		$P < 0.05$
vs. –5 min		NS	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$		$P < 0.05$
Saline	23.0	23.0	30.0	25.2	23.5	21.8		23.5
vs. –5 min		NS	$P < 0.05$	NS	NS	NS		NS
	–5 min	0 min	1 min	5 min	10 min	30 min	60 min	
Relative TBF								
^{32}P-CP	1.00	1.00	1.35	1.03	0.89	0.82	0.75	
vs. saline	NS	NS	$P < 0.05$	NS	$P < 0.05$	$P < 0.05$	$P < 0.01$	
vs. –5 min		NS	NS	NS	$P < 0.05$	$P < 0.05$	$P < 0.01$	
Saline	1.00	1.00	0.83	0.97	0.95	1.05	1.08	
vs. –5 min		NS	$P < 0.05$	NS	NS	NS	NS	

*Median value; n = 6 mice per group. ^{32}P-CP treated tumors were compared to the control (0.9% saline group) at each time point. Either TIFP or TBF obtained at various time points were compared to their initial values (values at 5 min prior to the starting of treatments) in the same tumors. For each comparison, Wilcoxon's two-tailed test was applied (NS, not significant).

saline did not change TBF. However, TBF decreased within 10 min after an i.t. injection of ^{32}P-CP, reaching 75% ($P < 0.01$) of control value by 1 h (Table IV).

DISCUSSION

The first goal of this study was to evaluate the cellular uptake or adsorption of ^{32}P-CP on the exponential and plateau growth-phases of our chosen tumors in vitro. In T-25 cell culture flasks plated with no cells, the passive adsorption of ^{32}P-CP to plastic was ~20%. The maximum uptake of 10 μCi of ^{32}P-CP using T-75 cell culture flasks plated with $HepG_2$ human hepatoma cells, reported by Order et al. [12], was ~1.8 μCi per aliquot, which was ~20%. However, in this study, the uptake or passive adsorption in 24 multiwell plates was <1%, presenting accurate and reliable results. The uptake of this radionuclide-labeled colloid was also dependent on both the stage of tumor growth and on tumor cell lines. In Ls174T tumors, the uptake of ^{32}P-CP in plateau growth-phase was close to two times more than that in exponential growth-phase (Table I).

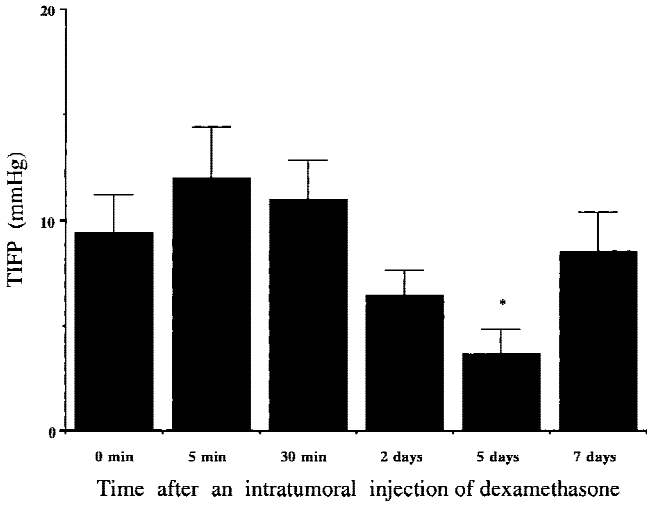

Fig. 1. Changes in TIFP after an intratumoral infusion of dexamethasone in Ls174T tumors (n = 7) in nude mice. Median TIFP values with interquartile ranges (bars) are shown. The asterisk represents statistically significant ($P < 0.05$) by applying two-tailed Mann-Whitney test.

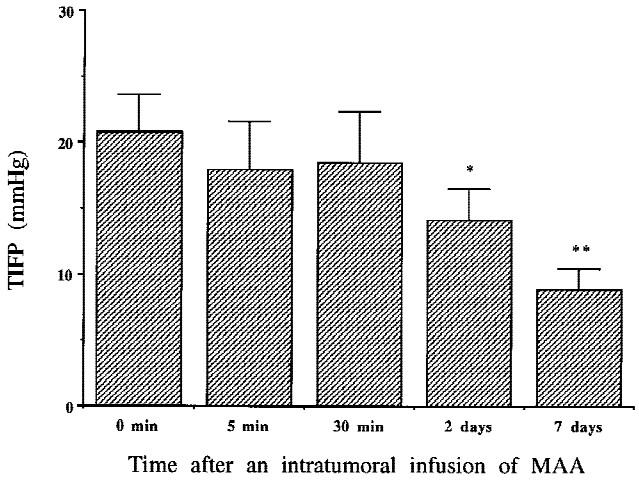

Fig. 2. Changes in TIFP after an intratumoral infusion of MAA (100 μl infusion volume of 10^6 particles) in AsPC-1 tumors (n = 7) in nude mice. Median TIFP values with interquartile ranges (bars) are shown. "*" and "**" represent statistically significant (*P < 0.05; **P < 0.01) by applying two-tailed Mann-Whitney test.

It is well agreed that ~90% of radioactive colloids remained in the liver, when Levine et al. [4] infused via the portal or hepatic artery. This was mainly due to high adherence of ^{32}P-CP. Therefore, if ^{32}P-CP is introduced intratumorally (one way to overcome the tumor hypertension), it should maintain highly efficient tumor targeting, due to the high adherence of ^{32}P-CP to the infused regions.

When degradable starch microspheres (DSM) were introduced intraarterially, TBF decreased immediately due to the transient embolization. However, TBF returned to the original level within 40 min [16]. The tumor sizes were also significantly decreased after five repeated DSM treatments. If they had measured both TBF and TIFP, it would have clarified whether DMS transiently embolized either the arterial or venous sides of the tumor vasculature. We observed that MAA did not kill any tumor cells or modify the cell viability in vitro. Also, the i.t. injections of MAA did not shrink tumor volumes in vivo (unpublished results, Lee and Wallner, 1996).

MAA labeled with ^{99}Tc has been used in nuclear medicine practices for the detection of pulmonary emboli, due to the particular size of MAA [17]. The median MAA size is ~5 times bigger than the diameter of a red blood cell [18]. Therefore, if ^{99}Tc-MAA were injected into the hepatic artery as reported by Sigurdson et al. [19], regional chemotherapy may be enhanced due to the temporary MAA-induced blockage of the outflow vasculature. However, this hepatic MAA-infusional trial still could not overcome the physiological barrier in solid tumors, the tumor hypertension [5,6]. We observed that both Ls174T and AsPC-1 tumor had significantly elevated TIFP, as shown in Table II.

Two tendencies between TIFP and tumor volume were also observed: one is increasing TIFP with increasing tumor volume (i.e., FSaII murine fibrosarcoma, HGL-9 human glioma), and the other is constant TIFP with increasing tumor volume [8,20]. We previously reported that the TIFP in Ls174T tumors did not increase with increasing tumor volume [20]. However, in AsPC-1 tumors, TIFP significantly increased as a function of tumor volume between 100 and 2,000 mm^3. Furthermore, a positive relationship between TIFP and water content in FSaII tumors was documented [8]. As a result, water content as measured by ^{1}H-NMR could be utilized to estimate TIFP noninvasively [21].

The second goal of this investigation was to evaluate the physiological response after i.t. infusion of various materials: ^{32}P-CP, MAA, albumin, and dexth. When the outflow of the tumor vasculature was obstructed by an i.t. infusion of materials (in the absence and presence of MAA) or mechanical occlusion, TIFP significantly increased and remained at those levels. However, we did not observe any increased TIFP due to the MAA-induced transient blockade of tumor vasculature, as shown in Table III. Consequently, the transient blockage of the outflow by an i.t infusion of MAA, the current major topic in the ongoing clinical trials, may not be a plausible mechanism in human cancer treatments.

We observed that blue-colored MAA (labeled with trypan blue) remained in the small infused sites 1–3 days after an i.t. infusion of various volumes (50 to 200 μl) and particle numbers of MAA (10^6 to 10^8 MAA particles/ml) in both Ls174T and AsPC-1 tumors. As we speculated, due to the larger sizes of the MAA particles (about five times larger than red blood cells), they could not travel through the entire tumor region after an i.t. infusion into the blood vessel. In contrast, the trypan blue solution (50–100 μl) spread homogeneously blue throughout the entire region. Hence, an i.t. injection of a large infusion volume containing targeting materials can improve the delivery of small molecules into the entire tumor region. However, this preclinical trial showed that an i.t. infusion of MAA prior to an i.t. infusion of trypan blue reduced the improvement of homogeneous distribution in the delivery of trypan blue. Instead of the ideal homogeneous distribution with an entirely blue tumor, there were many "spots" of blue instead, which indicated that MAA was still inside the tumor regions.

An i.t. injection of ^{32}P-CP significantly reduced TBF in AsPC-1 tumors, reaching 75% of control value by 1 h (Table IV). However, we did not observe any MAA-induced reductions in TBF. It was previously documented that, using clamping methods, when the arterial inflowing vessels were clamped, both TIFP and TBF significantly decreased to near zero or to background levels [22]. When the clamp was released, these parameters returned to the original levels [14]. However, when the venous outflowing vessels were clamped, the TIFP

significantly increased, but TBF decreased. Therefore, this study showed that the successful tumor targeting of ^{32}P-CP can be achieved without additional i.t infusion of MAA, due to the transient reduction in TBF by this therapeutic colloid. One of the main components of ^{32}P-CP is dextrose (i.e., aqueous suspension in a 30% dextrose solution). As a result, the high concentration of dextrose present in the colloid significantly increased the viscosity, therefore reducing the TBF.

CONCLUSIONS

This intralesional ^{32}P-CP brachytherapy may offer a promising treatment modality delivering high doses of tumor selective radiation in a relatively short period of time in an outpatient setting. The therapeutic ratio may be further increased by fractionation of intralesional ^{32}P-CP brachytherapy, mainly due to two physiological mechanisms: the high adherence of ^{32}P-CP to the infused regions, and reduction in TBF by this therapeutic colloid. There is a need for further investigation of fractionated ^{32}P-CP intralesional brachytherapy; for example, by developing animal models using orthotopical tumors such as tissue-isolated human ovarian tumors grown in the ovaries of nude mice [23].

REFERENCES

1. Kaplan WD, Zimmerman RE, Bloomer WD, et al.: Therapeutic intraperitoneal ^{32}P: Clinical assessment of the dynamics of distribution. Radiology 1981;138:683–688.
2. Jackson GL, Blosser NM: Intracavitary chromic phosphate (^{32}P) colloidal suspension therapy. Cancer 1981;48:2596–2598.
3. Condra KS, Mendenhall WM, Morgan LS, et al.: Intraperitoneal P-32 for adjuvant and consolidative therapy in ovarian carcinoma. Int J Radiat Onc Biol Phys 1996;36(Suppl.):328.
4. Levine B, Hoffman H, Freedlander SO: Distribution and effect of colloidal chromic phosphate (P^{32}) injected into the hepatic artery and portal vein of dogs and men. Cancer 1957;10:164–171.
5. Jain RK, Baxter LT: Mechanisms of heterogeneous distribution of monoclonal antibodies and other macromolecules in tumors: Significance of interstitial pressure. Cancer Res 1988;48:7022–7032.
6. Jain RK: Delivery of novel therapeutic agents in tumors: Physiological barriers and strategies. J Natl Cancer Inst 1989;81:570–576.
7. Jain RK: Barriers to drug delivery in solid tumors. Sci Amer 1994;271:58–65.
8. Lee I, Boucher Y, Jain RK: Nicotinamide can lower tumor interstitial fluid pressure: Mechanistic and therapeutic implications. Cancer Res 1992;52:3237–3240.
9. Lee I, Boucher Y, Demhartner TJ, et al.: Changes in tumour blood flow, oxygenation and tumour interstitial fluid pressure induced by pentoxifylline. Br J Cancer 1994;69:492–496.
10. Lee I, Demhartner TJ, Boucher Y, et al.: Effects of hemorrhagic hemodilution and resuscitation on tumor interstitial fluid pressure, blood flow, and oxygenation. Microvasc Res 1994;48:1–12.
11. Order SE, Siegel JA, Lustig RA, et al.: Infusional brachytherapy in the treatment of non-resectable pancreatic cancer: A new radiation modality (preliminary report of the phase I study). Antibody Immunoconj Radiopharm 1994;7:11–27.
12. Order SE, Siegel JA, Lustig RA, et al.: A new method for delivering radioactive cytotoxic agents in solid cancers. Int J Radiat Oncol Biol Phys 1994;30:715–720.
13. Order SE, Siegel JA, Principato R, et al.: Selective tumor irradiation by infusional brachytherapy in nonresectable pancreatic cancer. Int J Radiat Oncol Biol Phys 1996;36:1117–1126.
14. Lee I, Wallner PE: Evaluation of cellular uptake, tumor retention, radiation response, and tumor pathophysiology in experimental solid tumors after an intratumoral infusion of colloidal ^{32}P-CP. Cancer 1997;80:2611–2617.
15. Lee I, Song CW: The oxygenation of murine tumor isografts and human tumor xenografts by nicotinamide. Radiat Res 1992;130: 65–71.
16. Yoshikawa T, Kokura S, Oyamada H, et al.: Antitumor effect of ischemia-reperfusion injury induced by transient embolization. Cancer Res 1994;54:5033–5035.
17. Renowden SA, Dunne JA, Hayward MWJ: Changes in arterial oxygen saturation during isotope perfusion scans using human macroaggregates of albumin. Nucl Med Comm 1991;12:959–963.
18. Bernard EJ, Nour R, Butler SP, Quinn RJ: Incidence of pulmonary embolism in single segmental mismatch on lung scanning. J Nucl Med 1994;35:1928–1931.
19. Sigurdson ER, Ridge JA, Kemeny N, et al.: Tumor and liver drug uptake following hepatic artery and portal vein infusion. J Clin Oncol 1987;5:1836–1840.
20. Boucher Y, Lee I, Jain RK: Lack of general correlation between interstitial fluid pressure and oxygen partial pressure in solid tumors. Microvasc Res 1995;50:175–182.
21. Steen RG: Edema and tumor perfusion; characterization by quantitative ^{1}H NMR imaging. Am J Roentgenol 1992;158:259–264.
22. Wiig H, Gadeholt G: Interstitial fluid pressure and hemodynamics in a sarcoma implanted in the rat tail. Microvasc Res 1985;29: 176–189.
23. Kristjansen PEG, Roberge S, Lee I, et al.: Tissue-isolated human tumor xenografts in athymic nude mice. Microvasc Res 1994;48: 389–402.